

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

**Re:** Patent Application for Nyhus  
**Serial No.:** 09/811048 (49)  
**Filed:** March 15, 2001  
**For:** Inbred Corn Line G4901  
**Dated:** March 4, 2004  
**Art Unit:** 1638  
**Examiner:** Ashwin Mehta  
**Action:** *Substitute*  
**AFTER FINAL RESPONSE**

**To:** Commissioner for Patents, P.O. Box 1450, Alexandria, VA 22313-1450

Please enter and make the amendments to the specification and claims as attached hereto.

**In the Specification:**

In the Specification on page 18, line 10, please delete "#8".

**Remarks**

This is in response to the office action dated 12/19/2003, which was final. The examiner has indicated that claims 1-5 and claim 20 are allowable as written. The applicant believes that claim one is in better form as it is now amended; claim 2 has been amended to include its parts. This amendment does not appear to change the scope of the protection. And claim three has been amended to insert a comma that was missing between ovule and anther. Claim 20 has been amended to delete "the". This amendment does not alter the scope of the claim it only acts to place it in better form for allowance. The newly added claims are believed to find full support in the specification and are believed allowable as written. The applicant has added these claims as most were indicated as allowable in other cases. The newly added claims are not believed to require any additional costs. However, if there are additional costs for any claims or an extensions of time is needed the cost should be withdrawn from Account No. 07-0190.

**Claim Objections**

The Examiner objected to claims 13, 14, 15, and 17 under 37 CFR §1.75 (C) as having improper form because a multiple dependent claim should refer to other claims in the alternative only. The claims have been deleted. And replaced by similar claims

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directed to the method of making the maize inbred with a desired trait (see claim 18 and claim 31 for the altered starches) and a method of making a maize plant with a transgene that confers at least one selected resistance (see claim 12). The examiner indicated in the office action that inserting amylose extender instead of ae was new matter. The Applicant's attorney is supplying 4 pages of article ( three articles in total) one of the articles is dated 1997 to show that ae and amylose extender are the same thing and that the ordinarily skilled person in the art of corn breeding would know that AE is a mutated gene that is referred to as amylose extender. The Applicant's attorney offered to provide the Examiner with an affidavit to this effect but the Examiner indicated that it was not necessary if the articles showed ae was in use.

Therefore, all of the objections the examiner had to the claims should now be removed.

#### **Claim Rejections – 35 USC §112**

The examiner found that the claim language in 12 and 15 was indefinite as the plant of claim 2 was not transgenic. Claim 12 has been amended in accordance with language that the office appears to find clearer. The office has allowed similarly written claims but they were not written in the Markush style. It is believed that claim 12 as amended should be allowed. If the Examiner feels that the claim should be written as separate claims and not in the Markush style the applicant would appreciate a phone call to that effect. However, the metes and bounds of claim 12 is now believed to be clear

#### **Deposit Requirement**

The examiner has agreed to hold the deposit requirement in abeyance. When the client has made a decision to pay the issue fee based on allowable claims, then the Applicant will deposit seed.

#### **Written description**

Claim 12 as amended is believed allowable as now written.

There is sufficient written description for the removed claims in the Applicant's attorney's opinion. Still in an effort to further this prosecution, the applicant's attorney has deleted claims 6-11 and 13-17 and amended claim 12 and claim 18 to clearly stay

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within the parameters of what the examiner considers adequately described subject matter. Thus the remaining claim 12 and 18 as now written should be allowable as written.

The applicant would like it clear on the record that claims that were amended or cancelled were not necessarily amended or cancelled in a manner that would suggest that the claims are not allowable.

The applicant's attorney kindly requests that the examiner reconsider the claims as now amended and grant allowance of these claims.

Respectfully submitted,



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## # 6 DISEASE RESISTANCE IN INBRED G4901

Eye Spot = 5.1

Gross wilt = 4

Northern leaf blight = 1.0

## 5 #7 INSECT RESISTANCE IN INBRED

ECB1 = 6.75

ECB2 = 4.76119

Ear rate = 3.50000

10 **[#8]** The comparable inbred to G4901 is ZS01595, an inbred having a number of similarities. ZS01595 is an inbred which has been or is presently in a number of commercial hybrids that are in a similar region of adaption as most of the hybrids formed with G4901.

15 The Munsell code is a reference book of color, which is known and used in the industry and by persons with ordinary skill in the art of plant breeding.

The purity and homozygosity of inbred G4901 is constantly being tracked using isozyme genotypes as shown in Table 2.

## 20 Isozyme Genotypes for G4901

Isozyme data were generated for inbred corn line G4901 according to procedures known and published in the art. The data in Table 2 gives the electrophoresis data on G4901 as compared to its two parents.

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**CERTIFICATE OF MAILING UNDER 37 C.F.R. 1.6**

I hereby certify that the foregoing RESPONSE AFTER FINAL OFFICE ACTION( three pages of claims 1-31, one of specification and the response (4 pages) and the 4 pages of articles on "ae" of 12/19/03 for application 09/811,045 is faxed to 703-872-9306 at Commissioner for Patents, P.O. Box 1450, Alexandria, VA / 22313-1450 on this 4<sup>th</sup> day of March 2004.

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Dana Wood



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## Abstracts

Publication no. C-1997-0612-04R | [VIEW ARTICLE](#)

### Thermal Properties of Corn Starch Extraction Intermediates by Differential Scanning Calorimetry (1).

F. F. Yamin (2), L. Svendsen (2), and P. J. White (2,3). (1) Journal Paper J-17130 of the Iowa Agriculture and Home Economics Experiment Station, Iowa State University, Ames, IA, Project no. 3128. (2) Student, assistant scientist, and professor, respectively, Dept. Food Science and Human Nutrition, and Center for Crops Utilization Research, Iowa State University, Ames, IA 50011. (3) Corresponding author. E-mail: [pjwhite@iastate.edu](mailto:pjwhite@iastate.edu) Phone: 515/294-3011. Fax 515/294-8181. Cereal Chem. 74(4):407-411. Accepted March 24, 1997. Copyright 1997 by the American Association of Cereal Chemists, Inc.

Thermal properties of corn starch extraction intermediates from four types of corn were studied using differential scanning calorimetry. Starch at four different stages of extraction, including a standard single-kernel starch isolation procedure and three starch extraction intermediates, was isolated from mature corn kernels of B73 and Oh43 inbreds and the mutants of waxy (*wx*) and amylose extender (*ae*) in an Oh43 background. Differences in thermal properties and moisture and protein contents of starch from the extraction stages were statistically analyzed. Most thermal properties (gelatinization and retrogradation onset temperatures, gelatinization and retrogradation ranges, gelatinization and retrogradation peak temperatures, gelatinization and retrogradation enthalpies, peak height index, and percentage of retrogradation) of starches extracted at stage 3 intermediate (a procedure that did not include a final washing step) were similar to those of starch extracted by the standard single-kernel isolation procedure. Values for gelatinization peak temperature, gelatinization enthalpy, and peak height index were different between the standard and the stage 3 intermediate. The values obtained from starches extracted at stage 3, however, were consistent and predictable, suggesting that this extraction intermediate might be used in screening programs in which many starch samples are evaluated. By using the stage 3 extraction, samples could be evaluated in three rather than four days and the procedure saved approximately equals 0.5 hr of labor time. The other two starch extraction intermediates, which excluded filtering and washing or filtering, washing, and steeping, produced starch with thermal properties generally significantly different from starch extracted by the standard single-kernel isolation procedure.

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03/03/2004

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Direct any comments  
to Dr. Charles Auer  
Last updated 10/26/03

## Evaluation of GEM Experimental Crosses for Starch Amylose

Nurtay Abdubeck

Dr. Mark Campbell, Faculty Mentor. GEM Cooperators'  
Meeting. American Seed Trade Association, 1999. Chicago, IL

Breeding for high-amylose corn starch requires a rapid analytical method for determining starch amylose so that generating wet chemistry values does not pose a major limitation in the volume of materials that can be screened. Two recently described methods for determining apparent amylose were examined and compared to an earlier described iodine-binding method using isolated starch (method 1). These methods included one based on near-infrared transmittance spectroscopy (NITS) (method 2) and another iodine-binding method involving the solubilizing of starch from ground whole corn with a DMSO-iodine solution (method 3). These methods were chosen because, aside from initial set up costs, they are relatively rapid and inexpensive to perform. The materials evaluated consist of 155 different exotic populations including various plant introductions and experimental materials generated from the Germplasm Enhancement of Maize (GEM) project. Crosses were made between these materials and a Corn Belt dent hybrid (OH43 x H99) converted with the amylose-extender (ae) allele. F3 ears, presumed to be homozygous for the ae allele based on visual selection of the seed from which they were planted, were then evaluated in order to identify possible modifiers of ae conditioning high starch amylose. A core set consisting of 155 samples were selected (on F3 ear per exotic cross) from a total of 1006 ear samples harvested which were all subjected to starch amylose analysis using the three methods. The NITS method showed poor correlation to method 1 ( $r = 0.88$ ) however NITS did appear to discriminate between samples having been converted to ae versus those having a normal or possibly segregating endosperm type. Method 3 showed a much better correlation with method 1 ( $r = 0.92$ ) and appeared to better discriminate among samples having apparent amylose AA values >65% from those at or near 55%. Results from this study suggest that NITS may be useful when a quick screening method is needed to discriminate mutant from non-mutant genotypes especially when visual identification is difficult. In addition, method 3 could be used to replace the more time-consuming method 1 when trying to identify high AA levels among ae genotypes even though some inconsistency was observed between the two methods. Finally, this study revealed that exotic germplasm may be an important source of modifiers to the ae allele since values as high as 70% AA were identified.



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## Abstracts

Publication no. C-2002-0211-05R | [VIEW ARTICLE](#)

### Comparison of Methods for Amylose Screening Among Amylose-Extender (ae) Maize Starches from Exotic Backgrounds.

M. R. Campbell (1,2), H. Yeager (1), N. Abdubek (1), L. M. Pollak (3), and D. V. Glover (4). (1) Division of Science, Truman State University, Kirksville, MO 63501. (2) Corresponding author: Phone: 660-785-4280. Fax: 660-785-7604. E-mail: [campbell@truman.edu](mailto:campbell@truman.edu) (3) USDA-ARS Corn Insect and Crop Genetics Research Unit, Agronomy Dept, Iowa State University, Ames, IA (4) Agronomy Department, Purdue University, West Lafayette, IN 47907. Cereal Chem. 79(2):317-321. Accepted November 25, 2001. Copyright 2002 American Association of Cereal Chemists, Inc.

Breeding for high-amylose corn requires a rapid analytical method for determining starch amylose so that generating wet chemistry values does not pose a major limitation in the volume of materials that can be screened. Two methods for determining apparent amylose content (AAC) were examined and compared with an iodine-binding method involving the solubilization of isolated starch in a sodium hydroxide solution (method 1). These methods included one based on near-infrared transmittance spectroscopy (NIRT) (method 2) and another iodine-binding method involving the solubilizing of starch from ground whole corn with a DMSO-iodine solution (method 3). These methods were chosen because, aside from initial set up costs, they are relatively rapid and inexpensive to perform. The materials evaluated consisted of various exotic corn populations including plant introductions and experimental materials generated from the Germplasm Enhancement of Maize (GEM) project. Crosses were made between these materials and a Corn Belt dent hybrid (OH43 × H99) converted with the amylose-extender (ae) allele. Grain from F2 ears, presumed to be homozygous for the ae allele based on visual selection of mutant kernels on F1 ears from which they were planted, were then evaluated to identify possible modifiers of ae conditioning high starch AAC. From a total of 1,006 F2 ears, a core set consisting of 155 samples was established and only these were subjected to starch AAC analysis, using all three methods to compare the methods. Method 2 showed poor correlation to method 1 ( $r = 0.88$ ), however, NIRT did appear to discriminate between samples converted to ae vs. those with a normal or possibly segregating endosperm type. Method 3 showed a slightly better correlation with method 1 ( $r = 0.92$ ) and appeared to more fully discriminate among samples with AAC values >65% from those at approximately 55%. Results from this study suggest that NIRT may be useful when a quick screening method is needed to discriminate mutant from nonmutant genotypes within grain samples of exotic germplasm especially when visual identification is difficult. In addition, method 3 could be used to replace the more time-consuming method 1 when trying to identify high AAC levels among ae genotypes, even though some inconsistency was observed between the two methods. Finally, this study revealed that exotic germplasm may be an important source of new modifiers to the ae allele because values as high as 70% AAC were identified.



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